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DOI: https://doi.org/10.1016/j.tvjl.2015.05.007

Short Communication

Detection of *Chlamydia pneumoniae* in a collection of captive snakes and response to treatment with marbofloxacin

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Highlights

- *Chlamydia pneumoniae* was detected in 8/53 (15%) snakes in a private collection in Switzerland.
- In one snake that died, chlamydiae were detected by immunohistochemistry in multiple histiocytic granulomas.
- There was no clear evidence that *C. pneumoniae* caused morbidity or mortality in other snakes.
- There was no clear evidence that treatment with marbofloxacin effectively cleared *C. pneumoniae* infection from snakes.

Abstract

In a collection of 58 snakes comprising predominantly Eurasian vipers in Switzerland, five snakes died unexpectedly during hibernation from 2009 to 2012. In one snake, organisms resembling chlamydiae were detected by immunohistochemistry in multiple histiocytic granulomas. Real-time quantitative PCR and microarray analysis were used to determine the presence of *Chlamydia pneumoniae* in tissue samples and cloacal/choanal swabs from snakes in the collection; 8/53 (15.1%) of the remaining snakes were positive. Although one infected snake had suppurative periglossitis, infection with *C. pneumoniae* did not appear to be associated with specific clinical signs in snakes. Of seven snakes treated with 5 mg/kg marbofloxacin IM once daily, five became PCR negative for *C. pneumoniae* following treatment, whereas one animal remained positive and one snake was lost to follow-up.

Keywords: *Chlamydia pneumoniae*; Snake; Diagnosis; Treatment; Marbofloxacin

Chlamydia pneumoniae was first described in humans in association with respiratory disease and has been reported in horses, marsupials, amphibians and reptiles (Roulis et al., 2013). Whilst much is known about the symptoms and treatment of *C. pneumoniae* in humans, there are few reports of chlamydiae in reptiles (Jacobson et al., 2004; Soldati et al., 2004; Frutos et al., 2014). *C. pneumoniae* has been identified in granulomatous lesions in squamata (Jacobson et al., 2004; Soldati et al., 2004), but the pathogenicity of this agent remains unclear**.** Here, we report the detection of *C. pneumoniae* in a private collection of snakes, comprising *Viperidae*, *Colubridae*, *Pythonidae* and *Elapidae*, along with the diagnostic and therapeutic protocol adopted.

Out of a total of 58 snakes in the collection, five (8.6%) died unexpectedly during hibernation from 2009 to 2012 (see Appendix: Supplementary Table 1). On pathological examination, a 2.5 year old female horned viper (*Vipera ammodytes ammodytes)* had multifocal histiocytic granulomas in the large blood vessels proximal to the cardiac atrium (Fig. 1a) and in the liver and splenopancreas. Occasional presumptive chlamydial inclusion bodies were detected in histiocytes in sections stained with haematoxylin and eosin, and the presence of *Chlamydia* was confirmed by immunohistochemistry using a *Chlamydiaceae* Family-specific monoclonal antibody directed against the chlamydial lipopolysaccharide (ACI-P, Progen Biotechnik; Fig. 1b) (Soldati et al. 2004). Samples of lung, heart, liver, splenopancreas, kidney and intestine were tested using a *Chlamydiaceae* Family-specific real-time quantitative PCR (qPCR) and *C. pneumoniae* was identified using an ArrayTube microarray (Borel et al., 2008). Chlamydial infection was postulated as most likely cause of death in this snake. The other four snakes were negative for *Chlamydiaceae* by qPCR and did

not have consistent lesions at postmortem examination (see Appendix: Supplementary Table 1).

Choanal/cloacal swabs from six snakes that had either direct or indirect (fomite) contact with the index case were positive for *C. pneumoniae* by qPCR-microarray. The remaining 47 animals in the collection were also tested. In total, *C. pneumoniae* was detected in choanal/cloacal swabs from 8/53 (15%) live snakes (Table 1). All samples were negative by virus isolation and PCR for paramyxoviruses and adenoviruses (Blahak, 1995). Four months after the first screening, the eight *C. pneumoniae* positive snakes, along with one previously negative snake sharing a cage with a positive animal, and one snake that was positive for a Chlamydiaceae (not *C. pneumoniae*), were retested by qPCR-microarray. While 3/8 previously positive snakes became *C. pneumoniae* negative, the previously negative snake sharing a cage with a positive animal became positive for *C. pneumoniae*.

Marbofloxacin was selected in an attempt to eradicate *C. pneumoniae* from the collection, since quinolones are used frequently in reptiles and have been shown to be 100% inhibitory for *C. pneumoniae* in vitro (Hammerschlag and Roblin, 2000). The seven remaining positive snakes were treated with 5 mg/kg marbofloxacin (Marbocyl FD, Vétoquinol AG) IM once daily (Table 2). Choanal/cloacal swabs were tested for chlamydiae by qPCR at the onset of treatment and every 5 days until day 35. Treatment was discontinued as soon as all snakes in the same terrarium became qPCR negative or no later than day 35 for all the other snakes, since hibernation was scheduled for day 95. At day 35, 3/7 treated snakes were negative by qPCR for all *Chlamydiaceae*, one snake remained positive for *C.*

pneumoniae, one snake was positive for *Chlamydiaceae* (not *C. pneumoniae*), no sample was collected from one animal and one snake was sold.

After hibernation (day 320), the entire collection ($n = 45$; no samples from two animals, 12 animals sold, one animal dead due to egg retention and seven new acquisitions) was retested by qPCR-microarray. The snake that had been positive for *C. pneumoniae* at day 35 of treatment tested negative for *Chlamydiaceae* after hibernation; this snake had a suppurative inflammation of the tongue sheath at the first day of therapy, which eventually healed. The snake that was positive for *Chlamydiaceae* but negative for *C. pneumoniae* on day 35 remained positive after 320 days. All other snakes in the collection were negative by qPCR for chlamydiae ($n = 44$).

In summary, 5/7 snakes infected with *C. pneumoniae* became negative when treated with 5 mg/kg marbofloxacin IM once daily for 35 days. Evidence of persistent shedding of *C. pneumoniae* in one snake despite treatment suggests that marbofloxacin might not be entirely efficacious for treatment of chlamydial infections in reptiles. The suppurative inflammation of the tongue sheath in one snake healed during marbofloxacin treatment and the animal became negative by qPCR.

Previous studies (Jacobson et al., 2004; Soldati et al., 2004) found inclusion bodies in granulomas from dead snakes and identified chlamydiae by electron microscopy and immunohistochemistry, respectively; chlamydial DNA was also detected by PCR. In our investigation, *C. pneumoniae* was detected in 1/5 snakes that died, while none of the other eight animals that were positive for *C. pneumoniae* died. These findings suggest that *C.*

pneumoniae infections might not be necessarily lethal for snakes and are consistent with another report of clinically inconspicuous carriers (Frutos et al., 2013). This is further supported by the three cases in which infection with *C. pneumoniae* appeared to have resolved without antibiotic treatment. Clinical chlamydiosis in reptiles may be related to stress due to capture and transportation (Jacobson et al., 2004), high-density farming (Homer et al., 1994; Huchzermeyer et al., 2008) or, as speculated in the present study, hibernation.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgements

This study was supported, in part, by the annual grant 2013 from the Swiss Association for Wildlife, Zoo and Exotic Pet Medicine. The authors wish to thank the owner of the collection for his important contribution to this project, and Prof. Jean-Michel Hatt, Dr. Willi Häfeli and Dr. Silvia Blahak for their valuable comments.

Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi: …

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Figure legends

Fig. 1 (a) Histopathology of the index case (*Vipera ammodytes ammodytes*) that was positive for *Chlamydia pneumoniae* by immunohistochemistry, real-time qPCR and Arraytube microarray. Large blood vessels close to the atrium of the heart are obliterated by multifocal histiocytic granulomas (*) and thrombosis. Haematoxylin and eosin staining. (b) Immunohistochemistry for *Chlamydiaceae* antigen in a histological section of the heart of the index case, with red-brown granular immunostaining in the cytoplasm of histiocytes within a

182 granuloma in the wall of a major heart vessel (*). 3-amino-9-ethylcarbazole (AEC)-
183 peroxidase method with haematoxylin counterstain.
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Table 1

Live snakes screened by real-time quantitative PCR (qPCR) for *Chlamydiaceae* and ArrayTube microarray for *Chlamydia pneumoniae*.

			Number positive by qPCR-microarray		
Species	Female	Male	Choana	Cloaca	Both
Atheris chlorechis	3	1			
Atheris nitschei	2	1			
Bitis caudalis	2	2			
Cerastes gasperettii	1	1			
Elaphe climacophora	2	1			
Lampropeltis mexicana thayeri	1	1			
Montivipera bulgardaghica	2	1		1	
Montivipera xanthina	2	3			
Morelia bredli	1	1			
Morelia viridis	1	3			
Naja naja	1	1			
Pantherophis guttatus	1	3			
Vipera ammodytes ammodytes	3	3	2		3
Vipera ammodytes ruffoi	1	1	1		
Vipera kaznakovi	2	2			1
Vipera transcaucasiana	1	2		(1*)	
Totals	26	27	3	1 (1)	4

The sample marked with * was qPCR positive for *Chlamydiaceae*, but the species could not be identified by microarray. Snakes were fed with freshly killed mice from the owner's breeding colony. Basking areas (35 °C) and cool zones (room temperature 20-25 °C) were maintained on a 12 h/12h circadian cycle, while relative humidity was set according to the species' needs. All Eurasian snakes were hibernated in aerated styropore boxes in a refridgerator (Liebherr WK/GWK 611) at 8 °C and 70% relative humidity from 1 December to 1 March.

Table 2

Test and treatment protocol for seven snakes infected with *Chlamydia pneumoniae*.

			Day								
Species	Sex	Location	0	5	10	15	20	25	30	35	320
Vipera ammodytes	M	Choana	+	-	-					-	-
		Cloaca	-	-	-					-	-
Vipera ammodytes	F	Choana	+	-	-					-	-
		Cloaca	-	-	-					-	-
Vipera ammodytes	M	Choana	+	+ [a]							
		Cloaca	+	+ [a]							
Vipera ammodytes	M	Choana	+	+	-					-	-
		Cloaca	+	+	-					+ [b]	+ [b]
Vipera kaznakovi	M *	Choana	+	+	-						-
		Cloaca	+	+	-						-
Vipera ammodytes	F	Choana	-	-	-		-	-	+ [b]	-	-
		Cloaca	+	+	+	+	+	+	+	-	-
Vipera ammodytes	F [c]	Choana	+	+	+	+ [b]	-	-	-	-	-
		Cloaca	+	+	+	+	+	+	+	+	-

The presence of *Chlamydiaceae* was determined by qPCR and *C. pneumoniae* was confirmed by ArrayTube microarray. Empty cells indicate that no test was performed. Snakes were treated with 5 mg marbofloxacin IM once daily (grey shading) from day 1 until tested negative or to day 35 if they remained positive. Hibernation was imposed from day 95 to day 320.

[a] Snake was sold.

[b] Chlamydial species could not be identified by Arraytube Microarray.

[c] This snake had suppurative inflammation of the tongue sheath at the first day of therapy. None of the other snakes exhibited clinical signs.

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